EFFECT OF HIGH PRESSURE ON MOISTURE AND NaCl DIFFUSION INTO
TURKEY BREAST

A Thesis

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ABSTRACT

High pressure processing (HPP) has shown to affect the mass transfer of components into food. The effect of pressure (0.1-300 MPa) on the diffusion of NaCl and water into turkey breast meat processed at 25°C was evaluated. Turkey sample suspended at 50g/L NaCl solution held at 25°C served as control. NaCl and water diffusion were found to be a function of pressure and holding time. During the pressure come-up-time diffusion of both water and NaCl into turkey breast was enhanced as compared to control. At 150 MPa, the sample moisture diffusion coefficient was found to be minimal, while NaCl diffusion coefficient was maximum. Application of pressure, NaCl and water diffusion altered meat textural properties. Hardness, gumminess and chewiness of sample increased with pressure holding time and were minimal at 150 MPa. Cohesiveness was not affected with pressure application, but was found to increase with holding time. Examination of meat ultrastructure revealed that 150 MPa pressure treatment resulted in swelling of myofibrils, disappearance of the M-line, reduced difference in the density of the A-band and I-band and breaking of segments of Z-line. However, the myofibrils structure was intact, which led to the increased water holding capacity. HPP could be a useful technique for the salting of turkey breast meat, which may help in reducing the processing time as improving the textural properties.

Dedicated to my parents Johnny Villacís and Celeste Barraqueta, my sisters -Ximena and Lorena, and my brothers - Juanjo and Marcelo

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CHAPTER I

INTRODUCTION

1.1 Introduction

During the last decade, high pressure processing (HPP) has emerged as one of the commercially viable technologies to produce safe foods with fresh-like characteristics. This has given the industry the opportunity not only of producing products with better quality, but also of exploring processing new products which are otherwise difficult if not impossible to process. However, some of the effects of HPP to foods are not completely understood, especially those concerning transport phenomena (heat transfer, mass transfer). Mass transfer in foods is affected by product and process related factors. The product related factors include shape, size, composition, structure of the food, chemical and structural changes in the food during the process, among others. Since food is a complex heterogeneous material, these factors vary widely among different food materials; and thus determination of the mass transfer is a complex process.

Knowing how the diffusion occurs in food facilitates the process design as well as control of relevant unit operations. Addition of substances such as salt, phosphates, seasonings, among others to meat products has been done in the industry in order to incorporate flavor and improve the texture of the products. This is done by soaking the

meat in the mass transfer solution for several hours (still-marination). Alternatively it may be possible to tumble the meat in the solution or inject solution within the meat. Some of these processes may take long time and may increase of the risk for contamination.

1.2 High Pressure Processing

Although the possibility of using HPP to inactivate food microorganisms was known since the 19th century, this technology has been commercially used only during the last two decades. Increased consumer demand for food products with a high quality, fresh tasting properties as well as improved equipment capability facilitated this development. High-pressure food pasteurization use a pressure level around 400-600 MPa with or without heat. Sterilization implies the use of elevated pressures (>500 MPa) and high temperatures (90-120°C). Most of the treatments require 3 to 10 minutes processing holding time. The application of these levels of pressure, temperature and time could cause alterations in the structure of the food which also would affect the transfer mechanisms.

1.3 Application of High Pressure Processing

Currently, the principal goal for the utilization of HPP is the production of fresh-like, microbiologically safe products. Products already available in the commercial market include guacamole, fruit jellies, jam, fruit juices, ice cream, salad dressings, salsa, rice products, seafood such as shucked oyster meat, and meat products such as tenderized meat, pork sausages, among others. From early studies, it is known that HPP can enhance

the osmotic dehydration of several fruits and vegetables. However, chemical and physical alterations into the food due to the application of high pressures may affect the diffusion process and the diffusion rates.

1.4 Effect of the addition of salt on meat

Sodium chloride is added to meat with different purposes and in different ways. NaCl can be added by immersing the meat in highly concentrated ($>5\text{M}$) NaCl solutions producing the elimination of water from the tissue and the incorporation of NaCl. This lowers the water activity extending the shelf life of that meat. Another way is the incorporation of brine into the meat. Relatively low concentration solutions ($\leq 1.5\text{ M}$), brines, are added to meat, enhancing the moistness and juiciness of the product by increasing the water holding capacity of the myofibrils in the tissue. However, different technologies have different impact on the tissue structure modifying the water holding capacity.

1.5 Problem Statement

Currently limited literature is available to characterize the mass transfer in different substances subjected to pressure treatment. Food products could go through cell damage when pressurized, which could alter the diffusion behavior. Further, chemical and structural changes facilitated because of the presence of the diffusing substance or the high pressure application may significantly alter the diffusion rate. Thus, in order to design different process under this new technology, it is necessary to investigate the effect of HPP on the diffusion of compounds in food under controlled process conditions.

Recently, the meat industry has been interested in using HPP to produce meat products. In order to produce meat products under this technology, it is necessary to understand and be acquainted with the characteristics of the different involved mechanisms under different conditions of pressure, time and temperature. Brining of meat by traditional methods (atmospheric pressure still-marination, tumbling) leads to a process which can take several hours.

1.6 Objectives

This research was conducted with the following objectives:

- Determine the effect of high pressure processing on the water and salt diffusion into turkey breast muscle
- Calculate effective diffusion coefficients during high pressure processing of meat samples
- To study the textural changes in the turkey muscle suspended in brine solution subjected to high pressure processing.

CHAPTER 2

REVIEW OF LITERATURE

2.1 High Pressure Processing

High pressure processing is an alternative preservation technology that can be used for food pasteurization and sterilization. The technology has been demonstrated to reduce the number of microorganisms, destroy pathogens and inactivate deteriorative enzymes, extend shelf life while maintaining product quality attributes such as color, texture, and flavor in most food products (Fellows, 2000; Knorr, 1999). During HPP, the food material is typically pre-packaged, and subjected to pressures (100-800 MPa) with or without addition of heat.

The basic principles of high pressure processing process are (Earnshaw, 1996):

- Le Chatelier's principle which states that, under equilibrium conditions, a process which involves the increase in the pressure will favor those changes involving a decrease in volume.
- Isostatic principle which states that pressure is transmitted in a uniform and quasi-instantaneous manner throughout the whole product.

The advantages of using high pressure processing are:

- Shelf life extension is typically three times longer than thermally processed foods for high-acid foods (Polydera, Stoforos, & Taoukis, 2003) and two times longer for low-acid foods (Ramirez-Suarez, J. C., & Morrissey, M. T., 2006);
- Minimal effect on texture, fresh flavor and high quality;
- Rapid process; pressure accelerates microbial inactivation rates.
- High consumer acceptance

The limitations of using high pressure processing are (Earnshaw, R. 1996):

- High cost, low throughput
- Variable effect on enzymes and,
- Batch process & low throughput

2.1.1 High Pressure Equipment

Industrial scale high-pressure equipment is available either as batch or semi-continuous systems (Knorr, 1999). While pumpable products can be processed in a semi-continuous system, batch systems can be used for processing both solid and liquid pre-packaged products. Pre-packed food products are loaded into a basket and placed inside the pressure vessel (Fig 2.1). The vessel capacity ranges from few milliliters for research scale equipment to 600 liters for commercial scale production equipment. The vessel is filled with pressure transmitting fluid (water or oil based solution). The chamber is closed with help of the upper closure and pressurized with the intensifier pump. The desired pressure is maintained for the targeted holding time to be immediately decompressed. After decompression, the vessel is open and the food unloaded (Balasubramaniam, Ting, Stewart & Robbins, 2004).

Temperature, pressure and process holding time are controlled for each cycle. Because of the compression heating, product temperature increases with pressure (Fig 2.2). Temperature is maintained during the holding time if there is no heat loss to the environment. Holding time vary from 5 – 10 minutes depending of the product and the goal of the process. During depressurization the product temperature falls back close to its initial temperature.

2.1.2 Pressure treated products

High pressure pasteurized products are commercially available in USA, Europe, Japan, Australia and Mexico and Canada. Wide range of product categories including fruits and vegetables, sea-food, ready-to-eat meats, juices, and dairy products are pressure treated. Table 2.1 shows examples of high pressure processed products available in various part of the world

2.2 Mass Transfer

Mass transfer in foods has been extensively studied because it helps to predict, control, and design processes which involve the movement of components from one part of a system to another. Mass transfer plays an important role in a number of unit operations such as dehydration, extraction, smoking, baking, frying, drying, distillation, membrane separation, and absorption. Mass transfer may also be involved in a number of other processes such as salting, sugaring, deaeration, crystallization etc.

Figure 2.1: Simplified Schematic diagram of a high pressure processor.

A: Initial product temperature
 B: Temperature after pressurization
 C: Temperature before pressurization
 D: Temperature after depressurization

Figure 2.2: Sample Pressure – Temperature history during HPP.

Country (year)	Product	Process	Packaging	Shelf-life	Achievements of HPP & comments
Spain (1998)	Delicatessen: Cooked sliced ham and “tapas” (pork and poultry cuts)	400 MPa 10 min at 8°C	Darfresh vacuum-packed and gas-packed	2 months	Sanitization without color and taste modifications
USA (2001)	Cooked sliced ham, pork meat products and Parma ham		Vacuum-packed		Sanitization without color and taste modifications. <i>Listeria</i> destruction.
USA (2001)	Poultry ready-to-eat products		Gas-packed plastic pouches		Sanitization without color and taste modifications. <i>Listeria</i> destruction.
USA (2002)	Spicy sliced precooked chicken and beef for fajitas		Vacuum-packed	21 days	Sanitization without color and taste modifications. <i>Listeria</i> destruction. The Fajitas kit is made of HPP meat but also HPP onions, peppers and guacamole.
Spain (2002)	Thick sliced ham, chicken and turkey products	500 MPa 4 to 10 min at +8°C	Vacuum packed Darfresh	2 months for cooked products	Sanitization without color modifications. <i>Listeria</i> destruction. Increase of shelf-life and additives reduction.
Italy (2003)	Porma ham (Prosciutto), salami, mortadela	600 MPa 10 min at +7°C	Vacuum-packed		Sanitization without color and taste modifications. <i>Listeria</i> destruction. Increase of shelf-life. Products for USA and Japan exports.
Japan (2005)	Cooked pork meta products nitrites-free: ham, sausages and bacon	600 MPa 5 min at +5°C	Vacuum-packed		Sanitization. Increase of shelf-life.
Germany (2005)	Smoked German ham: whole sliced and diced products	600 MPa 2 min at +5°C	Vacuum-packed		Sanitization. <i>Listeria</i> destruction. Products for USA export.
USA (2005)	Deli meats, sliced meat products.			120 days under refrigeration	Sanitization. Reduction of sodium, and additives

Table 2.1. Commercially available pressure treated products (Source: www.nchyperbaric.com)

In many food process operations, mass transfer is usually the rate limiting factor compared to heat transfer. Diffusion is a mass transfer process and can be defined by the migration of a system component from one part to another due to a concentration gradient (Singh & Heldman, 2001; Crank, 1975).

2.2.1 Solutions to Fick's law

Diffusion phenomena under diverse conditions can be described by mathematical models based on Fick's second law, which defines the diffusion for unidirectional unsteady state (Crank, 1975; Rastogi, Raghavaro, Niranjana & Knorr, 2002):

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} \quad \dots(1)$$

Where C is the concentration of diffusing substance, x the space coordinate measured normal to the section, and D is the diffusion coefficient.

Solutions for steady and unsteady states of diffusion are available considering one or more directions and different shapes of the solid material (Crank, 1975). Solutions have been proposed for different solids shapes such as infinite flat plate, rectangular parallelepiped, and finite cylinder. Lerici, Pinnavaia, Dalla Rosa, and Bartolucci (1985) evaluated the influence of the shape in the osmotic dehydration of apple pieces. Sticks, slices, cubes and ring shapes were evaluated giving different values for each of them.

Crank (1975) provided mathematical solutions for diffusion equation to estimate diffusion coefficients during different scenarios. Consider the case of diffusion of solute from a stirred solution of limited volume to a plane sheet. Assume that the solution is

well stirred, concentration of solute in the solution depends on the time and the total amount of solute in the solution and sample remains constant during the diffusion; Crank (1975) suggested the following solution:

If $x = 0$ at the center

at $t = 0$ (initial condition, Fig. 3.a.):

$C = C_0$: initial concentration of the solute in the sheet at $-l \leq x \leq l$

with the boundary conditions

$$\frac{\partial C}{\partial t} = 0 \text{ at } x = 0 \text{ for all } t \quad \dots(2)$$

$$\alpha l \frac{\partial C}{\partial t} = D \frac{\partial C}{\partial x} \text{ at } x = \pm l \text{ when } t > 0 \quad \dots(3)$$

Where l is the half thickness of the sheet, α is the ratio of the volumes of solution and the sheet ($\alpha = a/l$), and a is the extension of the space occupied by the solution.

The solution for the above conditions has been solved by Crank (1975), and presented the following equation for diffused solute ratio, M_r :

$$M_r = \frac{(m_t - m_\infty)}{(m_0 - m_\infty)} = \sum_{n=1}^{\infty} C_n \exp[-Dq_n^2 t/l^2] \quad \dots(4)$$

(a)

(b)

Figure 2.3. Diffusion of particles to and from a plane sheet in an enclosed chamber. (a) Initial condition before diffusion. (b) During diffusion. S: Diffusing solution. P: Plane sheet.

Where m is the solute content; the subscripts 0 , ∞ , and t represent the initial, equilibrium and concentrations at any-time t respectively; D is the effective diffusivity of the solute; the q_n 's are the non-zero positive roots of:

$$\tan q_n = -\alpha q_n. \quad \dots(5)$$

and

$$C_n = \frac{2\alpha(1+\alpha)}{(1+\alpha+\alpha^2 q_n^2)} \quad \dots(6)$$

Rastogi and Raghavaro (2004) developed a solution for a rectangular parallelepiped of sides $2a$, $2b$ and $2c$, giving the equation:

$$M_r = \frac{(m_t - m_\infty)}{(m_0 - m_\infty)} = \sum_{n=1}^{\infty} C_n^3 \exp \left[-Dq_n^2 t \left(\frac{1}{a^2} + \frac{1}{b^2} + \frac{1}{c^2} \right) \right] \quad \dots(7)$$

If all the sides of the parallelepiped are equal ($2a=2b=2c$), the solution is reduced to a cubical configuration (Rastogi et al., 2004)

$$M_r = \frac{(m_t - m_\infty)}{(m_0 - m_\infty)} = \sum_{n=1}^{\infty} C_n^3 \exp \left[-Dq_n^2 t \left(\frac{3}{a^2} \right) \right] \quad \dots(8)$$

Fig. 2.4. Element of volume used for mass transfer determination

2.2.2 Mass Transfer in foods

Different factors affect the mass transfer in a system. The presence of agitation during the process accelerates diffusion rate (Oztan & Mehmet, 2005). The concentration of solute in the diffusing solution affects significantly the diffusion behavior. Different concentrations have been considered in studies (Nicoletti, Romanelli, Gabas & Romero, 2003; Ünal, Erdoghd, Ekiz, & Ozdemir, 2004; Rastogi et al., 2004). Higher the concentration in the diffusing solution the faster the diffusion due to presence of concentration gradient between the two phases of the system. Likewise, the temperature alters the diffusion coefficients. In processes such as osmotic dehydration, it is expected that diffusion is greater when the temperature is higher (Rastogi et al., 2002); however in some other processes the utilization of high temperatures also involved chemical changes in the food which could slow down the diffusion of a solute on it (Ahromrit, Ledward, and Niranjana, 2006). Since the mass transfer occurs through the surface area exposed to the solution, the food size and shape also plays an important role. Diffusion process time

increases with sample size as it dependant upon diffusion path length. pH and presence of other solutes can also influence diffusion.

2.2.3 Mass Transfer in Meats

Salting, marination, drying are some of the common examples for mass transfer in meats. During drying there is a loss in the moisture content of the meat. The effective moisture diffusivity coefficient has been calculated for pork ham during drying (Gou, Comaposada & Arnau, 2004). Gou et al. (2004) stated that the effective moisture diffusivity coefficient was affected by the process temperature and NaCl/moisture gradient. Several researchers estimated mass diffusion coefficients of various compounds from water based solutions to meat samples at atmospheric pressure (Oztan et al., 2004; Gou, Comaposada & Arnau, 2003; Unal et al., 2004; Wang, Tang & Correa, 2000). Graiver, Pinotti, Califano, and Zaritzky (2006) studied the effect of the sodium chloride concentration in the brining solution on the direction of the mass transfer. Authors reported that low concentration of sodium chloride (<10%) resulted in diffusion of water and salt from the solution to the meat, known as salting-in. On the other hand, for high salt concentrations, salt diffused from the solution into meat while water diffused out from the meat to the solution. This process is called as salting-out.

Graiver et al. (2006) calculated the NaCl diffusivity coefficients as $6.0 \times 10^{-10} \text{ m}^2/\text{s}$ when a 3% NaCl solution was used. A similar study using Pantanal caiman muscle considered the effect of temperature, initial brine concentration and brine/muscle ratio on the diffusivity values of NaCl (Nicoletti et al., 2003). At 20°C, with 15% (w/w) initial

brine concentration, for a brine/muscle ratio 5, an effective diffusivity value of 5.9×10^{-10} m²/s for NaCl was reported.

Gou et al. (2003) reported the effective moisture diffusivity values for ham pieces brined for 10 days at different NaCl concentrations and temperatures combinations. Among the treatments, the diffusion was the highest when the meat samples was submerged in 5% NaCl solution at 26°C ($D_e = 7.4 \times 10^{-11}$ m²/s). Wang et al. (2000) developed a model for predicting the salt concentration in different points of a salmon filet at different rigor states at room temperature and atmospheric pressure. The values ranged from $0.9 - 1.5 \times 10^{-10}$ m²/s. The diffusion coefficient of nitrite in calf meat was 1.1×10^{-10} m²/s (Oztan, et al., 2005). These values were enhanced by the presence of sodium chloride in the diffusing solution (Pinotti, Graiver, Califano, Zaritzky, 2002). The diffusion coefficient of sodium tripolyphosphate solution, which was an additive with antioxidative, antimicrobial and color development properties, was equal to 1.8×10^{-9} m²/s (Unal et al., 2004). NaCl diffusion coefficient values were reported by Sabadini et al. (1988) who obtained a value of 1.94×10^{-9} m²/s when salt drying beef. Del Valle and Nickerson (1967) reported 1.18 to 1.45×10^{-9} m²/s as the values for NaCl diffusion coefficients.

Xiong and Kupski (1999) reported an innovative method to evaluate the diffusion of phosphate in chicken filets by the utilization of dyes; however this method did not quantify the amount of solvent infused in the product. Wang et al. (2000) noticed the importance of the fat muscle content on the diffusivity value: a high fat content slowed down the salt diffusion process. As seen mass transfer of different compounds in meats have been investigated under atmospheric conditions, however scarce information

Product	Diffusivity Coefficient(m^2/s)	Study	Conditions	Reference
Pork tissue	0.41×10^{-9} ($NaNO_2$)	Nitrite & nitrate salts diffusion	20°C	Pinotti et al. (2001)
Pantanal Caiman Muscle	1.63×10^{-10} ($NaCl$)	Salting kinetics	20°C; Brine muscle ratio 1: 5 Initial brine concentration: 15%	Nicoletti et al. (2003)
Pork ham	3.9×10^{-11} (water)	Effect of temperature & $NaCl$ content during drying	19°C; Salting solution: 5kg $NaCl$ /100 kg water	Gou et al. (2003)
Beef	$2.00 \times 10^{-9} - 5.47 \times 10^{-9}$ (Na tripolyphosphate (STP))	Phosphate diffusion in meats	20°C ; 4% STP (w/v): 40°B	Ünal et al. (2004)
Pineapple cubes	1.48×10^{-9} (water) 0.53×10^{-9} (sucrose)	Osmotic dehydration	30°C ; Solution: 40°B	Rastogi et al. (2004)
Pre-osmosed carrot cubes	9.26×10^{-10} (water)	Convective dehydration	55°C; sucrose solution	Singh & Gupta (2006)
Cheese	7.8×10^{-12} (water) 5.3×10^{-10} ($NaCl$)	Water and salt diffusion during cheese ripening	12°C; 70-80%RH	Simal et al. (2000)
Carrot	0.593×10^{-9} (water) 0.797×10^{-9} (solute)	Effect of gamma irradiation during osmotic dehydration	Irradiation doses: 6.0 kGy	Rastogi (2005)
Thai glutinous rice	$\approx 6 \times 10^{-11}$ (water)	High pressure induced water uptake	20°C; 300MPa	Ahromrit et al. (2004)

Table 2.2 Examples of mass diffusivity coefficients in food systems

is available on how the application of high pressures would affect the mass transfer of compounds in meats.

2.2.4 Mass transfer during advanced thermal and nonthermal processes

Several studies have been conducted to examine the role of mass transfer phenomena during application of novel food processing technologies. Wang and Sastry (1993) examined the effect of ohmic heating in the salt diffusion on potatoes. Rastogi, Esthiagi, & Knorr (1999) concluded that the application of high electrical pulse enhanced the mass transfer during the osmotic dehydration of carrots. Deumier, Trystram, Collignam, Guedider, and Bohuon (2003) observed that pulse vacuum treatment improved the mass transfer diffusion in poultry meat because through vacuum the air placed in meat tissue spaces is eliminated and are those spaces which are occupied by the infiltrated solution. The effect of high pressure processing on the diffusion phenomena in fruits (Rastogi & Niranjana, 1998) and vegetables (Sopananukul, Ledward, & Niranjana, 2002; Ramaswamy, Balasubramaniam, & Sastry, 2005, Ahromrit, et al., 2006) were studied. Rastogi et al. (1998) evaluated the influence of high pressure processing as a pretreatment of a posterior osmotic dehydration process on pineapple pieces. Generally, osmotic dehydration is a slow process. A number of techniques have also been employed to improve mass transfer rate during osmotic dehydration. Application of high pressure caused permeabilization of the cell structure. This phenomenon has been exploited to enhance mass transfer rates during osmotic dehydration of pineapple. Authors concluded that the application of high pressure as a pretreatment improved the mass transfer of sucrose in the pineapples. Up to 400 MPa, the mass diffusivity increased with pressure,

while beyond 400 MPa no significant increase was observed. The pressure applied incremented the cell disintegration index in the internal tissue layers, which facilitated the interchange of components.

Ramaswamy et al. (2005) reported the synergistic effect of pressure pretreatment and the hydration temperature on the water uptake behavior of navy beans. Although, the rates of hydration at atmospheric pressure after the high pressure pre-treatment were lower than those hydrations made completely at atmospheric pressure, pressure treated samples achieved final moisture within a shorter time.

Sopanangkul et al. (2002) investigated the mass transfer of sucrose that occurs in potato cylinders under pressure. These researchers concluded that pressurizing up to 400 MPa modified the tissue structure and enhanced diffusivity. Further pressure increases caused starch gelatinization which slowed sucrose diffusion. A similar study by Ahromrit et al. (2006) was done in rice using water as the diffusing agent. Ahromrit et al. (2006) stated that diffusivity was affected by both pressure and temperature. Between 20°C and 50°C, pressure produced an increase in the diffusion up to 200 MPa. In the same temperature range, higher pressures caused a lower diffusion. Further, a temperature higher than 60°C produced a decrease in diffusivity coefficient values. Starch gelatinization during elevated pressure-temperature combination might have reduced the diffusivity values.

2.3 Effect of Sodium Chloride on Meat

Sodium Chloride has been widely used in meat products due to its favorable effects on the meat quality including shelf-life extension, flavor enhancement and protein

solubilization. Product preservation is achieved by lowering the water activity and increasing the ionic strength; therefore less water is available for microbial activity. The ability of NaCl to solubilize proteins is essential to extract salt-soluble proteins such as myofibrillar proteins (myosin and actin) and sarcoplasmic proteins producing an increase in the muscle tenderness. Besides, the liberation of myofibrillar proteins helps in fat particle emulsification (Barbout, 2002; Smith, 2001; Aktas, Aksu, & Kaya, 2003).

Salting-in is a process where meat pieces are immersed in moderate to low concentration salt solutions so that meat gains more water (Fig. 2.5). The amount of water gained depends on factors such as pH, temperature, rigor status, salt solution concentration, presence of other additives, massaging, tumbling etc. Maximum water uptake is observed when the salt concentration is around the physiological ionic strength, approximately 0.8 to 1 M (Offer & Trinick, 1983). At this concentration the proteins assemblies inside the myofibrils get dissociated. This results in a lateral expansion of the myofibrils creating more space inside the myofibrils to hold more water (Offer & Knight, 1988). Further NaCl addition diminished the muscle water holding capacity progressively. Thus, the muscle swelling decreases and shrinkage of the myofibrils is present when concentration is more than 4 M (Offer et al., 1988). This phenomenon is known as salting-out (Fig. 2.6).

2.4 Effect of High Pressure Processing on Meats

Together with the inactivation of microorganisms, pressurization on meat produces alterations of its structure, color and texture. Those changes were possibly due to modification in enzymes, proteins and lipids (Rastogi, Raghavarao, Balasubramaniam,

Niranjan & Knorr, 2006; Cheftel & Culioli, 1997). The type and extension of those modifications depended on the target pressure, process temperature and pressure hold time (Ma & Ledward, 2004; Cheftel et al., 1997).

2.5 Pressure effect on the Myofibrillar proteins

The effect of the pressure on the myofibrillar structure of the meat has been extensively studied. High pressure processing modified the myofibril structure which in turn affected the myofibril functional properties. Those alterations were function of the target pressure, temperature and holding time (Jimenez Colmenero, 2002). High pressure processing produced the fragmentation of the myofibrils, reduced interactions between the connection and myosin filaments, and altered the endomysium structure (Kamiyama, Ikeuchi, Suzuki, Kim, Hayashi, & Ito, 2001). Since higher myofibrillar expansion should occur to produce higher water retention (Offer et al., 1988), pressure may be contributing to this by facilitating the interactions between protein and water.

One of the principal effects of the application of relatively low pressures (< 300 MPa) to meat was the increase in the solubilisation of the myofibrillar proteins (Cheftel et al., 1997; Macfarlane, 1974; Macfarlane & McKenzie, 1976). Macfarlane (1974) reported that pressurization of 150 MPa produced a higher solubility of ovine myofibrillar proteins in contact with a 0.5 M NaCl solution. This effect was dependent factors such as pH, temperature, salt type, concentration (Macfarlane et al., 1976). Similar results were found for rabbit skeletal myofibrils in which the pressure up to 300 MPa and the solubilisation of myofibrillar proteins have a sigmoidal relationship. The principal proteins solubilised those present in the thin filaments and M-protein (Suzuki, Suzuki, & Saito, 1991).

Figure 2.5. Salting-in in meat

Figure 2.6. Salting-out in meat

According to Suzuki et al. (1991) the increase of the salt concentration from 0.1M to 1.0M produced an appreciable increment in the percentage of solubilised proteins. With higher protein solubility capacity higher water – protein interaction was expected (Cheftel et al., 1997). Further increase in pressure levels caused a decrease in the solubility of the proteins present in the meat tissue (Cheftel et al., 1997; Goutefongea et al., cited by Cheftel et al., 1997). Angsupanich, Edde, & Ledward (1999) found that 300 MPa pressure treatment at room temperature, for 20 min caused denaturation of myosin in turkey breast and suggested that the myofibrillar proteins were stabilized by hydrogen bonds forming new structures. Some adverse effect on the meat products have been observed when pressures > 150MPa. Meat batters processed at > 200 MPa for 5 minutes had a lower cooking yield and toughened texture (Mandava, Fernandez, Juillerat, 1994). Authors thought denaturation of proteins as one possible reason.

The combination of pressure and heat produced the disruption of thick and thin filaments in the muscle. A non-reversible disaggregation of the myosin of thick filaments was one of the principal effects of the application of pressure and heat. Application of 100 MPa -60°C treatment made irreversible effect on the myofibrillar structure however the connective tissue was not influenced (Bouton, Ford, Harris, Macfarlane, & O'Shea, 1977; Ma et al., 2004).

The application of pressure on chicken and pork batters with added sodium chloride showed to produce a high water and fat binding capacity. Water loss was reduced by through the formation of less rigid gels but with better binding properties. The synergetic effect of salt and pressure was more appreciable when the processing

temperature was 70 to 80°C (Jimenez-Colmenero, Fernandez, Carballo & Fernandez-Martin, 1998).

With the exception of the earlier investigations made by Macfarlane (1974, 1976) on extracted proteins, the combined effect of salt and pressure have not been investigated in animal tissue. More information about this area would allow a better understanding about the different changes observed on meats under pressure.

2.6 Pressure effect on meat texture

Texture is a commonly investigated property on meat as affected by the pressure. Meat texture depended on both the myofibrillar structure and the connective tissue network (Bouton et al., 1977; Harris & Shorthose, 1988; Macfarlane, 1973). Combined 103 MPa-35°C treatment for 2 minutes in different beef and sheep muscles decreased in the Warner-Bratzler (W-B) shear force values and reduced cooking loss (Macfarlane, 1973). Macfarlane, Turner and Ratcliff (1976) also reported similar results for beef biceps femoris muscles subjected to 103 MPa treatment for 6 minutes. The authors suggested that the reduction was due to a higher myofibrillar disruption, however the muscle membrane was believed not damaged by the pressure treatment since a lower fluid loss was observed in pressure treated samples. The disintegration of the M-line can be related with changes in the texture of the meat (Macfarlane et al., 1976). The breakages at the Z line were related with the increase of tenderness of meat (Suzuki, Watanabe, Iwamura, Ikeuchi, & Saito, 1990).

The application of pressure <150 MPa had different results. According to Cheftel et al. (1997), treatments less than 150 MPa at near atmospheric temperature (<30°C)

caused changes in the meat but did not alter the tenderness. Macfarlane et al. (1980) observed that texture after pressurization depends on the length of the sarcomeres. With shorter sarcomeres, the shear values increased due to pressurization, while long sarcomeres in the muscle resulted in higher tenderness. Macfarlane et al., 1978, reported that the sarcomere ultrastructure of stretched bovine *m. semimembranosus* was seriously affected by the application of 150 MPa pressure treatment at 30°C. Pressure level was enough to diminish the amount of myosin and induced connectin breakdown with the formation of low thermal resistant components.

Application of pressures greater than 150 MPa had a marked effect on different meat components including tenderness (Cheftel et al., 1997). Application of 200 MPa caused lower hardness in beef muscle when processed at 60 to 70°C (Ma et al., 2004). When a similar study was made in chicken, Zamri, Ledward, and Frazier (2006) reported that up to 50°C both heat and pressure synergistically affect the hardness of the muscle. At higher temperatures (60 – 70°C) the trend was unclear. The authors stated that the increasing hardness could be induced by the unfolding of myosin and actin, the formation of new structures and the compression of the thick filaments of the myofibrils. Suzuki, Homma, Kim, Ikeuchi, Sugiyama, and Saito (2001) reported that the conversion of α -connectin to β -connectin was almost complete at 300 MPa. Similar results were found by Kim, Ikeuchi, & Suzuki (1992) in pre-rigor rabbit *m. longissimus dorsi*, who also reported that nebulin was completely denatured at 200 MPa.

2.7 Pressure effect on the meat ultrastructure

The observation of the ultrastructure of the muscle gives an idea of how the effect of pressure was on the different section of the muscle. Besides, these alterations were related with other properties such as texture, water holding capacity and others. Suzuki et al., (1990) reported that high pressure processed bovine muscle presented no significant changes in its ultrastructure when treated up to 150 MPa for 5 minutes at 20°C. However, elevated pressures (>200 MPa) produced disappearance of the M-band. Similar results were reported by Macfarlane et al. (1978) who stated that this was the principal effect of the application of 100 MPa for 60 min at 25°C to ovine muscle. Similarly change in A band structure was reported, when subjected to pressures ≥ 200 MPa (Suzuki et al. 1990). This resulted in formation of longitudinal fissures. Suzuki et al. (1990; 1991) studied effect of pressure on beef and rabbit skeletal muscles. Application of 200 MPa resulted in near loss of continue sarcomere structure, breakages of A and I filaments, loss of M-line and thickening of Z-line. An application of 300 MPa produced a segmentation of the A-band. Breakage of the Z-line in the samples treated at 300 MPa was reported. The observations were consistent with those made by Macfarlane et al. (1978) in ovine muscle tissue. This rupture could be due to both the effect of the pressure and the salt. Macfarlane et al. (1978) suggested that the actin present in the Z-line may be altered by the application of pressure. On the other hand, the presence of salt produced a fragmentation of the Z-line (Offer et al., 1988). A lack of information which explains changes in the ultrastructure caused by the pressurization and salt diffusion is observed.

CHAPTER 3

EFFECT OF HIGH PRESSURE ON MOISTURE AND NaCl DIFFUSION INTO TURKEY BREAST

3.1 ABSTRACT

High pressure processing (HPP) affects food component diffusion. This study evaluated the effect of pressure (0.1-300 MPa) on the diffusion of NaCl and water into turkey breast meat processed at 25°C. NaCl and water diffusion were found to be a function of pressure and holding time. During the pressure come-up-time diffusion of water and NaCl into the sample was enhanced as compared to control. The diffusion coefficient of moisture infusing out of the sample was found to be a minimum at 150 MPa. The diffusion coefficient of NaCl infusing into the sample was a maximum value at 150 MPa. Hardness, gumminess and chewiness of sample increased with holding time and were minimal at 150 MPa. Ultrastructure images revealed that 150 MPa treatments caused swelling of myofibrils, disappearance of the M-line, reduced difference in the density of the A-band and I-band and breaking of segments of Z-line. HPP could be a useful technique for the salting of turkey meat.

Keywords: High pressure processing, Mass transfer, turkey breast, salting, ultrastructure, texture

3.2 INTRODUCTION

Salting of meat has long worldwide traditions as a preliminary operation in smoking, drying and cooking processes. This aids in enhancing product texture, flavor and shelf life (Smith, 2001; Graiver, Pinotti, Califano & Zaritzky, 2006). During salting process, two main simultaneous flows, NaCl and water movement, occur and the properties of muscle vary due to changes in the water and NaCl content. Diffusion of salt and water in meat takes place due to the differences in concentration and osmotic pressures between muscle cells and the salting agent.

When meat cuts are immersed in curing salts, salt penetration is related to the equilibrium between the interior salt concentration of the meat and the external brine solution. Diffusion of salt in meat has been widely studied (Djelveh & Gros, 1988; Dussap & Gros, 1980; Fox, 1980; Gros, Dussap & González-Méndez, 1984; Sabadini, Carvalho, Sobral & Hubinger, 1998; Wang, Tang & Correia, 2000; Wood, 1966).

Application of high pressure has been reported to accelerate the diffusion of components into the food. Pressure causes structural transformations in the food which may alter the diffusion coefficients. Rastogi and Niranjana (1998) have demonstrated that the application of pressure pretreatment resulted in increased rate of water and solute diffusion during osmotic dehydration of pineapples. Sopanangkul, Ledward, and Niranjana (2002) showed an increase in the diffusion of sucrose in potato cylinders up to 400 MPa, beyond which it decreased due to starch gelatinization. Ahromrit, Ledward and Niranjana (2004) studied the effect of high pressures on glutinous rice (or sticky rice) up to 600 MPa. Authors reported that the overall rates of water uptake and the equilibrium moisture content increased with pressure and temperature during soaking. Pressures below 300

MPa and temperatures below 60°C yielded higher effective diffusion coefficients. Above 300 MPa, the effective diffusion coefficient was not significantly dependent on temperature, which was attributed to starch gelatinization.

Application of high pressure processing (HPP) is being investigated in meat processing for microbial inactivation, meat tenderization, gelation or lipid oxidation (Lamballerie-Anton, Taylor & Culioli, 2002). Understanding transport mechanisms during HPP will facilitate the development of novel pressure applications and process optimization. However, to the best of our knowledge, no information is available in literature regarding the mass transfer behavior of meat under high pressures. The main objectives of this study are to a) determine the effect of high pressure processing on the NaCl and water diffusion in turkey breasts meat, b) estimate the corresponding diffusion coefficients and c) study the meat textural changes due to pressure treatment.

3.3 MATERIALS AND METHODS

3.3.1 Sample preparation

Turkey breasts from 140-days-old Commercial Tom Turkeys were procured in frozen conditions from a local poultry processor (Cooper Farms, Oakwood, OH). After slaughtering, the muscles were placed in the freezer and temperature of the breast reached -58°C within 72 hours, transported in frozen condition to the laboratory and maintained at -40°C. Before the experiment, the frozen turkey breasts were transferred at -10°C for 48 hours and then thawed at 4°C for 18 hours (Deumier, Trystam, Collignam, Guedider, & Bohuon, 2003). The turkey breasts were sliced using a meat slicer (model No. 1612, Hobart Slicer, Troy, OH, USA) and then cut in to cubes (20 x 20 x 20 mm). Samples

were equilibrated to 25°C for 15 minutes before processing. The pH of the sample was 5.85. The samples were immersed in 50g/L NaCl solution and vacuum packaged in high barrier bags consisting of Nylon/EVOH/ polyethylene (Winpak Ltd, Canada). The sample to solution ratio was maintained at 1:6. Turkey breast suspended in 50g/L NaCl solution held at 25°C served as the control sample.

3.3.2 High pressure processing

High pressure processing of turkey breast was carried out using a Quintus Food Processor (QFP-6, Flow Autoclave Systems, Columbus, OH). The pressure transmitting fluid was a 1:1 mixture of distilled water and food grade propylene glycol (Houghto-Safe 620-TY, Houghton International Inc., Valley Forge, PA). The rate of pressurization was approximately 258 MPa/min. The samples were processed at various pressures (50 to 300 MPa) for 1, 2, 3, 5, 10 and 15 minutes at 25°C $\pm$ 1°C. Similarly, pressure-come-up time effects were documented by processing the samples at various target pressures for 0.1 s. To achieve a temperature of 25°C during the pressure holding time, the initial temperature of the sample before HPP were lowered to compensate the rise in temperature because of compression heating during high pressure treatments (Ting, Balasubramaniam & Raghubeer, 2002). After processing, the pouch was cut open, samples were rinsed in distilled water, dried with a tissue paper to remove any residual water adhered to the surface, and subsequently packed in a plastic freezer bag, immediately air blast frozen at -20°C, and stored in the freezer at -20°C until analysis. The final pH of the samples treated under atmospheric pressure, 150 MPa and 300 MPa were 5.72, 5.68 and 5.79, respectively.

3.3.3 Moisture and NaCl determination

The moisture content of turkey breast pieces was determined using a Lab Wave 9000 moisture/solids analyzer (CEM Corp. Matthews, NC) following the AOAC 2000, Method No. 39.1.03. The values were expressed in kg of water/ kg of initial dry solids. NaCl content was determined by the modified Vohlrads method (AOAC 2000, Method No. 935.47). The NaCl concentration of each piece was expressed as kg of NaCl / kg of initial dry solids. The experiments were carried out in triplicate and average values were reported.

3.3.4 Texture profile analysis

INSTRON 5542 (Instron Inc. Canton, MA) with a 500 kg load cell was used to conduct texture profile analysis (TPA) of pressure treated and control turkey breast samples. The cubical sample (20 mm x 20 mm x 20 mm) were compressed (80% compression) on a non-lubricated platform using a flat disc probe (dia 38 mm), with a constant cross-head speed of 2 mm/s (Angsupanich & Ledward, 1998). Texture was measured perpendicular to the muscle fiber orientation. The peak force required to compress the samples were referred as a measure of hardness (Bourne, 1978). The cohesiveness, springiness, gumminess and chewiness were determined from TPA following the definition as described by Bourne (1978). Cohesiveness is expressed as the dimensionless quotient of the areas represented by the work to be done for two bites. Springiness (elasticity) is defined as the proportion of compression distance recovered between the first and the second compression. Gumminess is defined as the product of

hardness and cohesiveness, whereas chewiness is the product of hardness, cohesiveness and springiness. The data were collected for five replicates and average values are reported.

3.3.5 Statistical analysis

Statistical analysis was carried out using the SAS (SAS, release 9.2.1, SAS Institute Inc, Cary, NC, USA) software package to analyze the effect of pressure level, holding time on moisture, NaCl contents and meat textural properties. The general linear model and least significant difference procedures were used to compare means. Mean differences among pressure treatments and holding times were calculated by Duncan's multiple range tests.

3.3.6 Structure analysis

The ultrastructure changes in turkey breast muscle due to high pressure were evaluated using transmission electron microscopy (TEM). Six blocks of control and pressure treated tissue, each measuring 4 x 2 x 1 mm, were cut from the surface of anterior end turkey muscle and were subjected to a fixative solution consisting of 3% glutaraldehyde and 4% paraformaldehyde in 0.1M phosphate buffer containing 0.1M sucrose (pH 7.4) for 3 h at 20 °C. The fixed specimens were washed three times with 0.1 phosphate buffer containing 0.1 M sucrose. The samples were subjected to 1% OsO₄ for 1 hour and then rinsed three times with buffer solution and left overnight at 4°C. The samples were then dehydrated by immersing them in ascending concentrations of ethanol series (70%, 80%, 95% and 100%) for about 10 minutes each. This was followed by

washing for 10 min each consecutively in two changes each of 50:50% ethanol:propylene oxide (PO) and 100% PO. Both untreated and pressure treated specimens were then transferred into a vial containing PO: Spurr resin (1:1), and agitated in a rotator (Ted Pella Inc., Reading, CA, USA) for 1 hour. The solution was then replaced with PO: Spurr resin (1:2) and again agitated overnight. Resulting specimens were transferred into 100% Spurr resin in an embedding dish and held at 60°C for 24 h. Ultrathin microtome longitudinal sections (80 nm) were obtained on a Reichert Ultracut E ultramicrotome and mounted on grids and stained with 2% aqueous uranyl acetate followed by Reynolds lead citrate (Yoon, 2003; Jin, Zhu & Xu, 2006). Specimens were examined with FEI Technai Spirit transmission electron microscope (FEI Company, Hillsboro, OR, USA) operated at 80 kV. Photomicrographs were made at magnification of x 23,000.

3.3.7 Determination of water and NaCl diffusivities

The solution of Fick's second law for diffusion from a rectangular parallelepiped (sides 2a, 2b and 2c) result in the following well known equations for the transfer of water and solute, respectively (Crank, 1975; Rastogi et al., 1998; Rastogi, Raghavarao, Niranjana & Knorr, 2002; Rastogi & Raghavarao, 2004):

$$M_r = \frac{(m_t - m_\infty)}{(m_0 - m_\infty)} = \sum_{n=1}^{\infty} C_n^3 \exp \left[- (D_{ew})_p t q_n^2 \left(\frac{1}{a^2} + \frac{1}{b^2} + \frac{1}{c^2} \right) \right]; \quad \dots(1)$$

$$\text{and } S_r = \frac{(s_t - s_\infty)}{(s_0 - s_\infty)} = \sum_{n=1}^{\infty} C_n^3 \exp \left[- (D_{es})_p t q_n^2 \left(\frac{1}{a^2} + \frac{1}{b^2} + \frac{1}{c^2} \right) \right] \quad \dots(2)$$

where M_r and S_r are the moisture and NaCl ratio; m and s represent the moisture and solid content; the subscripts o , ∞ and t represent the relevant concentrations initially, at equilibrium, and at any given time; D_{ew} and D_{es} are the effective diffusivities of water and NaCl, respectively considering cubical configuration; and C_n is equal to $2\alpha(1+\alpha)/(1+\alpha+\alpha^2 q_n^2)$ where, q_n 's are the non-zero positive roots of the equation $\tan q_n = -\alpha q_n$. Here, α is the ratio of volume of solution to that of each piece.

When all the side of parallelepiped are equal ($2a = 2b = 2c$), then it reduces to a cubical configuration. Eqs. (1) and (2) reduces to:

$$M_r = \frac{(m_t - m_\infty)}{(m_0 - m_\infty)} = \sum_{n=1}^{\infty} C_n^3 \exp\left[-(D_{ew})_s t q_n^2 \left(\frac{3}{a^2}\right)\right]; \quad \dots(3)$$

$$\text{and } S_r = \frac{(s_t - s_\infty)}{(s_0 - s_\infty)} = \sum_{n=1}^{\infty} C_n^3 \exp\left[-(D_{es})_s t q_n^2 \left(\frac{3}{a^2}\right)\right] \quad \dots(4)$$

For a cubical configuration, Fourier numbers for moisture (F_{ow}) and NaCl diffusion (F_{os}) are defined as $D_{ew}t.(3/a^2)$ and $D_{es}t.(3/a^2)$, respectively (Rastogi et al., 2004). An approximate form of equations (3) and (4) (for cubical configuration) were graphically represented by plotting $\log(M_r \text{ or } S_r)$ against the Fourier number (Figure. 1). The slopes of these lines gave $d(\log M_r)/d(F_{ow})$ or $d(\log S_r)/d(F_{os})$.

The slopes of the experimental lines for moisture and NaCl diffusion [$d(\log M_r)/dt$ and $d(\log S_r)/dt$] were obtained by fitting the experimental data to the solution of mass transfer equations for moisture and NaCl mass transfer during salting process. The rate of change of moisture and NaCl content were plotted against average moisture and NaCl

content, respectively, and the equilibrium moisture and solid contents (m_{∞} , s_{∞}) as well as moisture and solid mass transfer coefficients (k_m , k_s) were estimated from these plots (Rastogi, Angersbach, Niranjana & Knorr, 2000).

Considering the equilibrium approach to mass transfer, the following equations for moisture and NaCl mass transfer can be written (Rastogi et al., 2000):

$$-\frac{dm}{dt} = k_m (m - m_{\infty}) \quad \dots(5)$$

$$\frac{ds}{dt} = k_s (s - s_{\infty}) \quad \dots(6)$$

where k_m and k_s are the moisture and NaCl mass transfer coefficients. Integration of Eqs. (5) and (6), with the appropriate initial condition, resulted in the following equations:

$$\ln \frac{(m_t - m_{\infty})}{(m_0 - m_{\infty})} = \ln M_r = -k_m t \quad \dots(7)$$

$$\ln \frac{(s_t - s_{\infty})}{(s_0 - s_{\infty})} = \ln S_r = k_s t \quad \dots(8)$$

The experimental data (M_r and S_r versus t) were fitted to Eqs. (7) and (8) to yield k_m and k_s , which in turn gave the slopes:

$$\frac{d}{dt}(\log M_r) = -\frac{k_m}{2.3025} \quad \dots(9)$$

$$\frac{d}{dt}(\log S_r) = \frac{k_s}{2.3025} \quad \dots(10)$$

D_{ew} and D_{es} values, considering a cubical configuration, were estimated from the following equations, respectively. (Perry, Green & Maloney, 1984; Rastogi et al., 2002):

$$D_{ew} = \left[\frac{d(\log M_r) / dt}{d(\log M_r) / dF_{ow}} \right] \cdot (a^2 / 3) \quad \dots(11)$$

$$D_{es} = \left[\frac{d(\log S_r) / dt}{d(\log S_r) / dF_{os}} \right] \cdot (a^2 / 3) \quad \dots(12)$$

3.4 RESULTS AND DISCUSSION

3.4.1 Salting of turkey breast at atmospheric pressure

The change in moisture and NaCl contents in the meat samples during salting at atmospheric pressure is shown in Figure 3.2. Product moisture and NaCl content increased with an increase in holding time. The increase in moisture content of meat after immersing in NaCl solution was due to swelling of the fibers and higher water holding capacity. The observations were similar to those reported by earlier researchers (Offer & Trinick, 1983; Medyski, Pospiech & Kniat, 2000; Graiver et al., 2006). The increase in water holding capacity might be attributed to the lateral expansion of myofibrils, which was coupled to protein solubilization. An increase in water binding and hydration in salted meat and muscle fibers were also attributed to enhanced electrostatic repulsion between myofibril filaments causing the filament lattices to expand for water entrapment (Xiong, Lou, Harmon, Wang & Moody, 2000; Pinotti, Graiver, Califano & Zaritzky, 2001). Akse, Gundersen, Lauritzen, Ofstad and Solberg (1993) reported that salting-in and swelling of the muscle occurred at lower NaCl concentration (<50 g/l), and no

salting-out of proteins was observed at this concentration. The values of mass transfer coefficients as well as diffusion coefficients of moisture and NaCl at atmospheric pressure were obtained and provided along with the equilibrium values in Table 3.1. The values of NaCl diffusion coefficients are in agreement with the values reported by Sabadini et al. (1988) and Del Valle and Nickerson (1967) in case of beef and fish salting, respectively. The negative sign of k_m under atmospheric pressure accounts for the direction of mass transfer that the water is diffusing from salt solution to the turkey breast.

3.4.2 Salting of turkey breast under high pressure

The plots for the variation of moisture and NaCl contents of pressure treated turkey breast sample immediately after the pressure come up time are presented in Figure 3.3. Pressure come-up time during high pressure treatment caused a significant amount of changes in moisture as well as NaCl content even though the starting material had the same moisture and NaCl content. Moisture and NaCl contents during pressure come-up time increased with increasing pressure (up to 150 MPa) and subsequent increase in pressure to 300 MPa resulted in decreased moisture and NaCl content ($p < 0.05$, Figure 3.3). This may be attributed to the increase in NaCl content with pressure treatment up to 150 MPa that resulted in increased water holding capacity. The observations were consistent with earlier reports (Offer & Knight, 1988; Gou, Comaposada & Arnau, 2003). Authors attributed that an increase in ionic strength due to NaCl diffusion solubilized the muscle protein, which in turn resulted in higher water holding capacity and tenderness. The pressures above 150 MPa resulted in decrease in solubility of protein, which may be

due to irreversible denaturation of F-actin in the absence of adenosine triphosphate (ATP) at a pressure around 150 MPa (Macfarlane & McKenzie, 1976) and complete denaturation was obtained at 300 MPa (Ikkai & Ooi, 1966). Ikeuchi, Tanji, Kim and Suzuki (1992) reported sulfhydryl content and hydrophobicity of F-actin were maximum at 150 MPa with further increases in pressure, these parameters decreased due to aggregation of actin.

The variation of moisture and NaCl content with pressure holding time during high pressure processing of turkey breast sample is presented in Figure 3.4a and 3.4b. Within the experimental conditions of the study, increasing pressure holding time increased NaCl content and decreased moisture content ($p < 0.05$). The moisture and NaCl content were always higher than the moisture and NaCl content of the untreated sample (Figure 3.3, 3.4a and 3.4b). These results were consistent with the observations made by Macfarlane (1974) that the immediate application of pressure resulted in increased high water holding capacity of the protein. Further, as the pressure holding time was increased the water holding capacity decreased due to increase in expressed soluble protein.

The moisture and NaCl contents for samples subjected to different pressures at the end of pressure-come-up time were different. Therefore, a simple plot of moisture and solid content versus time (Figure 3.4a and 3.4b) could not be directly used to compare mass transfer characteristics. Hence, the effective diffusivities of moisture (out of the sample), and solute (infusing into the sample) were determined. The relevant values (Table 3.1) indicated that the diffusion coefficient for moisture and NaCl decreased and increased, respectively up to 150 MPa; further increase in pressure to 300 MPa resulted

in increase in moisture and decrease in NaCl diffusivity values ($p < 0.05$). The changes in the cell structure and extent of protein denaturation due to high pressure application may probably be responsible for this observed effect. Thus at a given time, the sample treated at 150 MPa had a higher NaCl content and consequently gained more moisture than the samples treated at other pressures. The values of diffusion coefficients of moisture as well as NaCl under high pressure were always higher than the corresponding values at the atmospheric pressure. These observations are in agreement with the results reported by Sopanangkul et al. (2002) and Ahromrit et al. (2006) in case of sucrose diffusion in potatoes and water diffusion in rice, respectively.

3.4.3 Effect of high pressure treatment on textural properties

Textural properties of pressure treated turkey breast samples are shown in Figure 3.5. Hardness, gumminess and chewiness of sample decreased with an increase in pressure up to 150 MPa, further increase in pressure increased these values up to 300 MPa ($p < 0.05$). These results were in agreement with findings of Angsupanich et al. (1998). Authors reported that cod muscle textural properties decreased due to loss of myosin structure at lower pressure (up to 200 MPa). On the other hand, at higher pressures they were increased because of unfolding of actin and sarcoplasmic protein along with hydrogen bonded networks. On comparing the data in Figure 3.3 and Figure 3.5a, it may be noted that the hardness is linearly related to moisture content (or water holding capacity) of the product (Angsupanich et al., 1998).

Pressure holding time appeared to have influence on hardness, gumminess and chewiness of the meat samples. Cohesiveness and springiness did not change

significantly during the processing at different pressures ($p < 0.05$). However, the cohesiveness increased with pressure holding time for all pressures.

3.4.4 Effect of high pressure treatment on ultrastructure

The ultrastructures of the turkey breast muscles subjected to salting at different pressures and control are compared (Figure 3.6). The control sample had clearly defined M- and Z- lines; and around these lines H- and I- bands, respectively. The A-band was also seen between the two I-bands. The portion between the two Z-lines is called sarcomeres and these subunits are repeated along the length of the myofibril (Figure 3.6a). The salting at atmospheric pressure (0.1 MPa, Figure 3.6b) resulted in swelling of myofibrils. The treatment resulted in slight fading of M- and Z- lines. The H-band completely disappeared whereas the length of I-band significantly reduced.

At 150 MPa, swelling of myofibrils and disappearance of the M-line were seen. The difference in the density of the A-band and I-band were significantly reduced and broken segments of Z-line was also seen (Figure 3.6c). The length of sarcomere was found to be less in pressurized samples (150 MPa) as compared to control, which may be attributed to pressure-induced contraction (Harris & Shorthose, 1988; Suzuki, Watanabe, Iwamura, Ikeuchi, & Saito 1990). Elgasim and Kennick (1982) suggested that the thin filaments pull in towards the center of the A-band to the extent it shaded all the H-band area thus making it indistinguishable from rest of the area, which may be responsible for the reduction of the length of sarcomere. The beginning of the breakage of the Z-line at 150 MPa was probably due to the denaturation of F-actin, which was the principal component of the thin filaments connected to the Z-line (Ikeuchi et al., 1992). However,

the myofibrils structure was intact, which can hold higher amount of water. These results are in agreement with those of Suzuki et al. (1990) who studied the beef skeletal muscle.

The salting at 300 MPa resulted in significant changes in the structure of myofibrils (Figure 3.6d). The Z-line and I-band were completely destroyed and form a very dense re-aggregation of material around the location of the lost Z- and M-line. These changes may be responsible for the decrease in water holding capacity at higher pressures (>150 MPa).

3.5 CONCLUSIONS

Moderate pressure application (around 150 MPa) could be a potential technique for the salting of turkey breast meat, with improved textural properties. Pressure treatment of meat up to 150 MPa resulted in approximately 10-fold increase in NaCl diffusion coefficient, and thus potentially reduces the salting time in turkey breast sample. The textural properties such as hardness, gumminess and chewiness of sample were found to be minimal at 150 MPa. Examination of meat ultrastructure revealed that the myofibrils structure was intact, which led to the increased water holding capacity. The application of high pressure may potentially be used for the infusion of flavors and nutrients in the foods.

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Figure 3.1 Theoretical diffusion curve for cubical configuration as per
Eqs. (3) and (4)

Figure 3.2 Variation of moisture and salt content during salting of turkey breast at atmospheric pressure with treatment time

Figure 3.3 Effect of pressure come up time on moisture and NaCl content during salting of turkey breast at different pressures

Figure 3.4 Variation of (a) moisture and (b) NaCl content with holding time during high pressure processing of turkey breast sample.

Figure 3.5 Effect of Pressure on (a) hardness, (b) cohesiveness, (c) springiness, (d) chewiness, and (e) gumminess (▨ 0.1 s, ▨ 15 min holding time)

Figure 3.6 TEM of (a) control and pressure treated turkey breast sample during salting at various pressures (b) 0.1 MPa, atmospheric pressure; (c) 150 MPa; (d) 300 MPa. The samples b, c and d were subjected to 50g/L NaCl solution. The holding time for the sample subjected to at 0.1 MPa was 2h and for other samples was 15 minutes.

Treatment Pressure (MPa)	$D_{ew} \times 10^8$ (m ² /s)	$D_{es} \times 10^8$ (m ² /s)	k_m (min ⁻¹)	k_s (min ⁻¹)	m_∞	S_∞
0.1	0.28	0.52	-0.01	0.02	2.74	0.075
50	11.89	3.85	0.53	0.17	2.64	0.053
100	8.65	5.00	0.38	0.22	2.91	0.062
150	7.22	5.31	0.32	0.24	3.05	0.061
200	11.77	4.52	0.52	0.20	2.72	0.052
300	11.91	4.35	0.53	0.19	2.69	0.051

Table 3.1 Effective diffusion coefficients of water (D_{ew}) and NaCl (D_{es}) at different pressures

CHAPTER 4

CONCLUSIONS AND FUTURE RECOMMENDATIONS

4.1 Summary and Conclusions

Within the range of experimental conditions studied, application of high pressures accelerated the diffusion of both water and salt into the turkey breast pieces suspended in 50g/L brine solution, when compared with a similar process carried out at atmospheric pressures. Come up time has an important effect on the final content of NaCl and water into the turkey breast muscle. During come-up time, both moisture and NaCl contents increased with an increase in pressure, up to 150 MPa.

Holding time also influenced the uptake of salt and water in the turkey meat pieces. An increase in holding time resulted in a higher salt concentration in the sample. On the other hand, water content decreased during extended holding times. Final moisture content of the product was always higher than those values of the untreated sample.

Within the range of experimental conditions studied, maximum salt diffusivity coefficient was obtained at 150 MPa. At this same pressure, the effective moisture diffusivity coefficient out of the tissue was the lowest. Further increase in pressure up to 300 MPa produced still higher salt diffusion coefficients than the one at atmospheric pressure.

Similarly, pressure treatment at 150 MPa significantly influenced the meat textural properties. Hardness, gumminess and chewiness of sample decreased and further influenced by the magnitude of pressure holding time. These textural properties were affected by both the structure of the tissue and the higher concentration of water.

The analysis of the ultrastructure of the tissue under the different treatments improved our knowledge on the impact of the pressure treatment on the meat myofibrils structure. Other than the disappearance of the M-line, no significant change on the myofibril structure was observed when pressure up to 150 MPa was applied. Higher levels of pressure produced the complete destruction of the Z-line and I- band. This might have decreased the diffusion capacity of the tissue.

4.2 Future Recommendations

- Effect of HPP on the diffusion of NaCl and other components at different temperatures.
- Study the influence of pressure treatment on the water holding capacity and texture during storage and after cooking.
- Effect of pressures higher than 300 MPa on the ultrastructure of meat tissues.
- More research is required to understand the muscle protein modifications (solubility, precipitation, denaturation) occurred under high pressures and the presence of salts.
- Utilization of high pressure processing to facilitate the infusion of antioxidant and vitamins in different foods.

APPENDIX A

MATLAB PROGRAM FOR THE CALCULATION OF q_n AND C_n .

(Eqns. (4) & (5) (Chapter 3)).

Matlab Program for determining the positive roots of q_n and C_n . Values calculated for $n=1:6$ and $\alpha=6$.

```
%Q values when alpha is infinite taken from Crank (1975)
Q=[1.5708, 4.7124, 7.854, 10.9956, 14.1372, 17.2789];

'D =      n      qn      alpha      Cn'

for i=1:6
    q=Q(i);
    a=-tan(q)/(q);
    while a>6
        q=q+0.000001;
        a=-tan(q)/(q);
    end
    C=(2*a*(1+a))/(1+a+(a^2)*(q^2));
    R=[i,q,a,m];
    D(i,:)=R;
end
D
```

KEY:

$i = n$

$a = \alpha$

$q = q_n$

$C = C_n$

R= Results

Results in Matlab:

n	qn	alpha	Cn
1.0000	1.6703	6.0000	0.7819
2.0000	4.7475	6.0000	0.1026
3.0000	7.8751	5.9997	0.0375
4.0000	11.0107	6.0000	0.0192
5.0000	14.1489	5.9999	0.0116
6.0000	17.2884	5.9998	0.0078

APPENDIX B

MATLAB PROGRAM FOR THE CALCULATION OF m_∞ or s_∞ AND k_m or k_s .

Matlab Program for the calculation of M_{∞} or S_{∞} and k_m or k_s .

Example for calculation of s_{∞} and k_s when pressure = 50 MPa

```
%For 50MPa
load files50.txt
xdata=files50(:,1);
x1=files50(:,1);
ydata=files50(:,2);
guess=[5.26 .2];
options=statset('maxiter',1000);
[coefficient,r,J]=nlinfit(xdata,ydata,'crkk50',guess);
coefficient(1)
coefficient(2)
o=3.48;
    for t=[0:0.5:90];
        yhat=coefficient(1)+[(3.48-coefficient(1)).*(exp(-
coefficient(2)*t))];
        plot(t,yhat,'-r')
        hold on
    end
hold on
plot(x1,ydata,'*r')
```

ckk50.m:

```
function S=crkk50(guess,xdata);
a1=guess(1);
a2=guess(2);
%a1=s at infinite time
%a2=kvalue
x1=xdata(:,1);
S=a1+[(3.48-a1).*(exp(-a2*x1))];
```

KEY:

xdata = data for time

ydata = data for salt concentration

coefficient(1) = calculated value for salt concentration at infinite time

coefficient(2) = calculated value for k_s

a1= guessed value for salt concentration at infinitive time

a2= guessed value for k_s

APPENDIX C

TEXTURE PROPERTIES FOR PRESSURE AND NON-PRESSURE TREATED

TURKEY BREAST SAMPLES DURING SALTING

Time (min)	Pressure (MPa)					
	300	200	150	100	50	0.1
0.1	16.1 ± 1.5	14.7 ± 3.3	3.1 ± 0.2	6.0 ± 0.54	10.0 ± 3.1	11.0 ± 3.5
1	15.9 ± 2.9	15.3 ± 3.7	14.4 ± 2.0	13.7 ± 1.92	16.5 ± 3.4	12.5 ± 1.9
2	22.8 ± 3.1	20.8 ± 2.5	18.9 ± 1.1	16.6 ± 2.62	18.7 ± 3.2	13.7 ± 0.2
3	32.6 ± 3.0	28.2 ± 2.2	20.1 ± 2.5	20.5 ± 2.68	22.7 ± 0.9	13.4 ± 2.8
5	33.0 ± 0.9	31.8 ± 1.8	25.0 ± 1.2	24.2 ± 2.38	29.6 ± 3.1	14.3 ± 2.2

Table C.1. Hardness (N) of pressure treated and non-treated (0.1 MPa) turkey breast samples during salting.

Time (min)	Pressure (MPa)					
	300	200	150	100	50	0.1
0.1	4.3 ± 0.3	4.3 ± 0.7	4.5 ± 0.1	4.4 ± 0.7	4.0 ± 0.4	3.9 ± 0.6
1	4.3 ± 0.7	4.0 ± 0.8	4.4 ± 0.8	4.6 ± 0.6	3.6 ± 0.4	3.9 ± 0.3
2	4.6 ± 0.8	4.7 ± 0.1	4.5 ± 0.1	4.5 ± 0.3	4.3 ± 0.6	3.7 ± 0.3
3	4.3 ± 0.4	4.5 ± 0.8	4.4 ± 0.1	4.5 ± 0.7	4.0 ± 0.7	3.7 ± 0.1
5	4.2 ± 0.6	4.3 ± 0.1	4.4 ± 0.3	4.2 ± 0.6	3.9 ± 0.3	3.9 ± 0.3

Table C.2. Springiness (mm) of pressure treated and non-treated (0.1 MPa) turkey breast samples during salting.

Time	Pressure					
(min)	300 MPa	200 MPa	150 MPa	100 MPa	50 MPa	0.1 MPa
0.1	0.28 ± 0.06	0.28 ± 0.02	0.28 ± 0.01	0.26 ± 0.04	0.27 ± 0.08	0.34 ± 0.04
1	0.34 ± 0.08	0.32 ± 0.03	0.39 ± 0.03	0.38 ± 0.04	0.34 ± 0.03	0.35 ± 0.03
2	0.35 ± 0.02	0.37 ± 0.04	0.41 ± 0.02	0.38 ± 0.02	0.41 ± 0.03	0.35 ± 0.08
3	0.42 ± 0.05	0.40 ± 0.04	0.41 ± 0.03	0.39 ± 0.01	0.39 ± 0.01	0.31 ± 0.06
5	0.41 ± 0.02	0.40 ± 0.06	0.42 ± 0.03	0.41 ± 0.05	0.40 ± 0.03	0.34 ± 0.07

Table C.3. Cohesiveness of pressure treated and non-treated (0.1 MPa) turkey breast samples during salting.

Time	Pressure					
(min)	300 MPa	200 MPa	150 MPa	100 MPa	50 MPa	0.1 MPa
0.1	22.8 ± 4.6	15.4 ± 5.3	6.5 ± 1.0	6.9 ± 1.1	10.7 ± 5.2	14.4 ± 3.2
1	25.0 ± 5.0	19.6 ± 2.3	25.1 ± 3.2	25.9 ± 0.9	25.0 ± 2.7	17.5 ± 3.7
2	37.1 ± 5.8	36.9 ± 1.9	31.6 ± 9.1	33.9 ± 1.6	33.0 ± 4.3	18.2 ± 4.3
3	57.0 ± 3.7	51.2 ± 2.1	31.4 ± 6.6	38.8 ± 4.8	35.7 ± 4.9	16.0 ± 2.3
5	60.4 ± 5.7	55.8 ± 2.6	40.1 ± 0.6	41.6 ± 3.2	55.8 ± 4.8	19.3 ± 5.3

Table C.4. Chewiness (Nmm) of pressure treated and non-treated (0.1 MPa) turkey breast samples during salting.

Time (min)	Pressure					
	300 MPa	200 MPa	150 MPa	100 MPa	50 MPa	0.1 MPa
0.1	4.6 ± 0.4	4.0 ± 1.0	0.9 ± 0.1	1.6 ± 0.4	2.7 ± 1.6	3.6 ± 1.5
1	5.8 ± 2.2	4.8 ± 1.4	5.6 ± 1.6	5.2 ± 0.9	5.5 ± 0.7	4.4 ± 0.9
2	8.1 ± 1.3	7.9 ± 0.3	7.8 ± 0.4	6.3 ± 1.1	7.5 ± 1.8	4.9 ± 1.0
3	13.2 ± 0.7	11.4 ± 1.4	8.1 ± 0.9	7.9 ± 1.2	8.8 ± 0.2	4.3 ± 0.5
5	13.5 ± 0.5	12.6 ± 1.3	9.5 ± 1.1	9.6 ± 1.7	11.7 ± 1.2	5.0 ± 1.7

Table C.5. Guminess (N) of pressure treated and non-treated (0.1 MPa) turkey breast samples during salting.

APPENDIX D

SAS SYNTAX

SAS SYNTAX FOR DETERMINING SIGNIFICANT MEAN DIFFERENCES IN TEXTURE PROPERTIES AMONG DIFFERENT TREATMENT CONDITIONS

```
data turkey;  
    input pressure time @;  
    do i=1 to 3;  
        input y @;  
        output;  
    end;  
datalines;  
  
[DATA]  
  
proc glm;;  
class pressure time;  
model y = pressure time pressure*time /ss1 ss2 ss3 ss4;  
    lsmeans time pressure*time / stderr;  
    lsmeans pressure / stderr pdiff;  
run;
```

KEY

Pressure – refers to pressure conditions such as 100 MPa

time – refers to the pressure holding time such as 0.1 sec

i – refers to the number of replicates

APPENDIX E

TEM OF PRESSURE TREATED TURKEY BREAST SAMPLES

Figure E.1 TEM pressure treated turkey breast sample during salting at (a) 150 MPa for 0.1 seconds, (b) 300 MPa for 0.1 seconds. (c) Pressure treated turkey breast without salting at 150 MPa for 15 minutes.

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